REMARKS UNDER 37 CFR § 1.111

Formal Matters

Claims 69 and 71-88 are pending after entry of the amendments set forth herein.

Claims 69-88 were examined. Claims 69-88 were rejected. No claims were allowed.

Claims 69, 71, 84, and 87 are amended. Support for these amendments is found in the specification at, for example, page 5, line 29 to page 6, line 11. "Plurality" is amended to "mixture" simply for ease in antecedent reference in the body of the claim.

Claim 70 is canceled without prejudice.

Applicant respectfully requests reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added.

Overview of the Claimed Invention

The claimed invention is a method that provides for detection of a polymorphic nucleotide (e.g., a single nucleotide polymorphism (SNP)) in a polynucleotide in a sample, where the method involves primer extension using an extension reaction mixture comprising:

-- a primer that specifically hybridizes to a target sequence so that there is a one or more nucleotide gap between the 3' terminus of the primer and the variant nucleotide of the polymorphic site;

-- a mixture of dNTPs or rNTPs that provide for at least one nucleotide extension regardless of which of the two variant nucleotides is present at the polymorphic site

Importantly, the reaction mixture excludes:

-- a dNTP or rNTP complementary to one of said variant nucleotides

-- labeled dNTPs/rNTPs; and

-- ddNTPs.

The reaction products are then analyzed. The primers and dNTP/rNTP mixture are designed so that the length of the primer extension products is indicative of the identity of the variant nucleotides at the polymorphic site.

The method of the invention provides for certain advantages, such as:

- the cost of the method is reduced compared to conventional methods, since there is no requirement for either detectable labels or dideoxy nucleotides, which reagents are expensive
- the method provides that the dNTP/rNTP mixture and primer sequence are designed so that at least one nucleotide extension occurs regardless of which of the variant nucleotides is present at the polymorphic site so that the length of the primer extension products is indicative of the identify of the variant nucleotides at the polymorphic site.
    - the method thus provides an "internal control" for extension in that both the presence and the absence of a variant nucleotide can be detected in a single reaction – for example, if the variant nucleotide complementary to a dNTP in the mixture is absent, an extension product is formed nonetheless. Stated differently, the claimed method provides for detection of a "negative" result for a particular variant nucleotide.
    - this is in contrast to conventional methods, where the absence of the variant nucleotide complementary to a dNTP in the mixture results in no extension – one can not tell whether the extension reaction did not work or whether the variant nucleotide is not present
    - this same feature is useful in, for example, detection of whether a diploid sample is from a homozygous or heterozygous individual

We now turn to the outstanding rejections.

Interview Summary

Applicant is grateful to Examiner Souaya for her time and effort in preparation for an in-person interview with the undersigned, and her time and assistance during the interview on June 26, 2003. All outstanding rejections of the claims were discussed, and amendments and arguments proposed by counsel to distinguish the claims from the cited art and avoid the rejections under §112, ¶2 were discussed.

The Examiner and the counsel agreed upon language to avoid all rejections of the claims, which language is presented in the claim amendments submitted here.

Rejection under §112, ¶1

The claims were variously rejected as being indefinite. These rejections are addressed below.

A) Recitation of "nucleotides 5' of a variant nucleotide"

Claims 69-88 were rejected for recitation of "nucleotides 5' of a variant nucleotide". This rejection is avoided by amendment of the claims using the language kindly suggested by the Examiner. The claims now recite that the primer hybridizes to the target sequence such that there is a one or more nucleotide gap between the 3' terminus of the primer and the variant nucleotide of the polymorphic site of the target sequence. The primer thus is positioned so that the primer is extended by at least one nucleotide regardless of the identity of the variant nucleotide at the polymorphic site.

B) Recitation of "one or more nucleotides"

Claims 69-88 were rejected for recitation of "one or more nucleotides". This rejection is avoided by cancellation of claim 70 to clarify that primers that hybridize so that their 3' terminus are immediately adjacent to the variant nucleotide are excluded.

C) Recitation of "3' end"

Claims 69-88 were rejection for recitation of "3' end" of the primer. The claims are amended to recite "3' terminus" as kindly suggested by the Examiner.

In view of the above, withdrawal of the rejections of the claims under §112, ¶2 is requested.

Rejections under §103

The claims were variously rejected as being obvious under §103. These rejections are as follows:

1) Claims 69-74, 76, and 82-83 were rejected over Soderlund (EP 0648280) in view of Hoogendoorn and Kuppuswamy;
2) Claims 75 and 77-81 were rejected over Soderlund in view of Hoogendoorn and Kuppuswamy and further in view of Gibson; and
3) Claims 81, 82 and 84-88 were rejected over Soderlund in view of Hoogendoorn and Kuppuswamy and further in view of Krook

Thus in each rejection, Soderlund is cited as the primary reference.

In short, applicant respectfully submits that none of the references, either alone or combined, teach or suggest a method involving a primer extension reaction where 1) both ddNTPs and labeled dNTPs are omitted from the extension reaction; 2) the primer that specifically hybridizes to a

target sequence so that there is a one or more nucleotide gap between the 3' terminus of the primer and the variant nucleotide of the polymorphic site; and 3) the length of the primer extension products is indicative of the identity of the variant nucleotide at the polymorphic site.

We first discuss each of the references cited, and then consider the combined disclosure as applied to the claims.

Soderlund

Soderlund is cited for its disclosure of a method for detecting polymorphic nucleotides, particularly at Example 9 and in Fig. 1d. However, neither Example 9 nor any part of Fig. 1 – or any other portion of the Soderlund reference – teaches or suggests a method of that detection of the presence/absence of a variant nucleotide of a polymorphic site **without using 1) either a detectably labeled dNTP to detect primer extension (Kuppuswamy) OR 2) ddNTPs to terminate the reaction should a particular variant nucleotide be present in the target sequence (Hoogendoorn).**

Specifically, in Example 9, Soderlund indicates that the extension reaction is performed with dTTP in the reaction mix (see page 17, lines 8-11). In Fig. 1, either the dNTP is labeled or a ddNTP is present. Moreover, the primer is Fig. 1 hybridizes so that the 3' terminus is immediately adjacent the variant nucleotide, and complementary nucleotides were added to confirm the presence of the polymorphic variants.

The Office Action points to Soderlund at page 7, line 1 in support of the assertion that Soderlund teaches the use of ddNTPs is not required, and is an optional variation. However, the Office Action has not noted the context in which this statement is made. The relevant sentence from Soderlund states;

> **When a labeled dNTP is used**, it is advantageous, but not necessary, to add unlabelled ddNTPs corresponding to the other three nucleotide residues (option c in Fig 1).

(Soderlund, paragraph 57, page 7, lines 13-15, emphasis added).

From this statement in its entirety, it is evident that Soderlund only suggests that ddNTPs are optional when the dNTP is labeled. There is no teaching or suggestion that both the dNTP can be unlabeled and ddNTPs omitted as per the present claims.

With respect to the primer, the claimed method recites that the primer specifically hybridizes to a target sequence so that there is a one or more nucleotide gap between the 3' terminus of the primer and

the variant nucleotide of the polymorphic site. Soderlund states that such a primer may be used. However, in this context Soderlund also states:

> The detection primers can also be complementary to a sequence beginning several nucleotides removed from the variable nucleotide. The only limitation concerning the position of the detection step primers is that the sequence between the 3' end of the detection step primer and the variable nucleotide to be detected **must not contain a nucleotide residue of the same type as the one to be detected**.

(Soderlund, paragraph 23, page 3, line 57 to page 4, line 3, emphasis added). The claimed method avoids this technical challenge of Soderlund. Instead, the claimed method provides for the use of primers and dNTP (or rNTP) mixture so that extension products of different lengths are produced depending upon the identity of the variant nucleotide at the polymorphic site. Thus, the length of the primer extension products produced according to the claimed method is indicative of the identity of the variant nucleotides at the polymorphic site.

Hoogendoorn and Kuppuswamy

The disclosures of Kuppuswamy and Hoogendoorn have been discussed previously. The disclosure of these references with respect to the claimed method can be summarized as follows:

1) Kuppuswamy uses detectably labeled dNTPs, and is either silent as to the use of ddNTPs or does not require ddNTPs in view of the use of the labeled dNTPs
2) Kuppuswamy teaches that separate reactions should be performed for each variant nucleotide, and thus does not contemplate the design of the primer and dNTP mixture so that the identity of the variant nucleotides of the polymorphic site based upon the length of the extension products; and
2) Hoogendoorn requires the use of ddNTPs, and the length-based detection method of Hoogendoorn simply would not be operable without such ddNTPs.

Neither Kuppuswamy nor Hoogendoorn, either taken alone or in combination, suggest that detection of the presence/absence of a variant nucleotide of a polymorphic site can be detected **without using 1) either a detectably labeled dNTP to detect primer extension (Kuppuswamy) OR 2) ddNTPs to terminate the reaction should a particular variant nucleotide be present in the target sequence (Hoogendoorn).** The claimed method explicitly excludes the use of labeled dNTPs and ddNTPs. Furthermore, in contrast to the claimed method, neither Kuppuswamy nor Hoogendoorn

provides for the use of primers and dNTP (or rNTP) mixture so that extension products of different lengths are produced depending upon the identity of the variant nucleotide at the polymorphic site.

Gibson

Gibson is cited for its disclosure of separation of DNA fragment according to size using capillary electrophoresis. Gibson adds nothing to the disclosure of the Soderlund, Kuppuswamy, or Hoogendoorn with respect to performing primer extension in the absence of labeled dNTPs and the absence of ddNTPs, or to the use of primers and dNTP (or rNTP) mixture so that extension products of different lengths are produced depending upon the identity of the variant nucleotide at the polymorphic site.

Krook

Krook is cited for its disclosure of multiplexed or polled single nucleotide primer extension reactions using more than one primer for extension, where the primers are of different lengths. Krook teaches use of a labeled dNTP in the extension reaction. Krook thus does not cure the deficiencies of the disclosures of Soderlund, Kuppuswamy, or Hoogendoorn with respect to performing primer extension in the absence of labeled dNTPs and the absence of ddNTP's, or to the use of primers and dNTP (or rNTP) mixture so that extension products of different lengths are produced depending upon the identity of the variant nucleotide at the polymorphic site..

Combined disclosures

None of the cited art, either taken alone or in any combination, teaches:

- both the dNTP can be unlabeled and ddNTPs omitted from the reaction mixture as per the present claims.
- the 3' terminus of the primer is one or more nucleotides 3' of the variant nucleotide of the target sequence
- the use of primers and dNTP (or rNTP) mixture so that extension products of different lengths are produced depending upon the identity of the variant nucleotide at the polymorphic site.

Therefore, in view of the above, the claims are not obvious in view of any combination of the cited references, and this rejection can be withdrawn.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number TGEN-001.

Respectfully submitted,
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